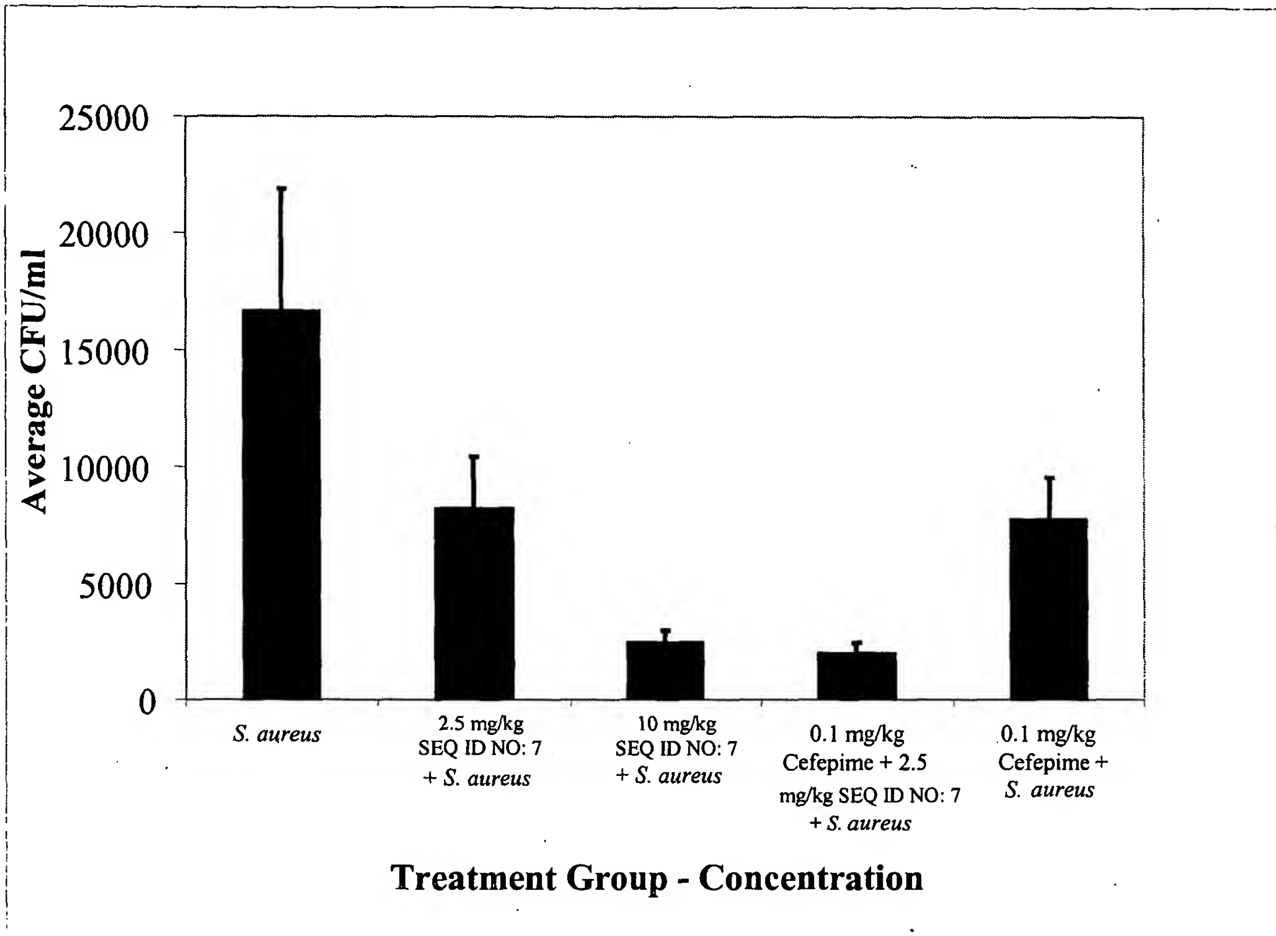


Figure 1: Synergy of Seq ID No: 7 with cefepime in curing *S.aureus* infections. CD-1 mice (8/group) were given 1 x 10^7 *S. aureus* in 5 % porcine mucin via IP injection. Test compound (50 µg – 2.5 mg/kg) was given via a separate IP injection 6 hours after *S. aureus*. At this time Cefepime was also given at a dose of 0.1 mg/kg. Mice were euthanized 24 hr later, blood removed and plated for viable counts. The average ± standard error is shown. This experiment was repeated twice.

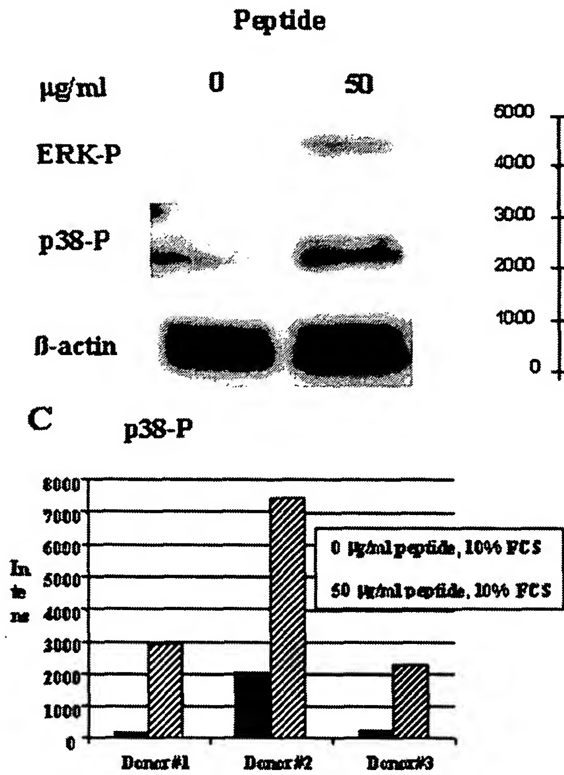
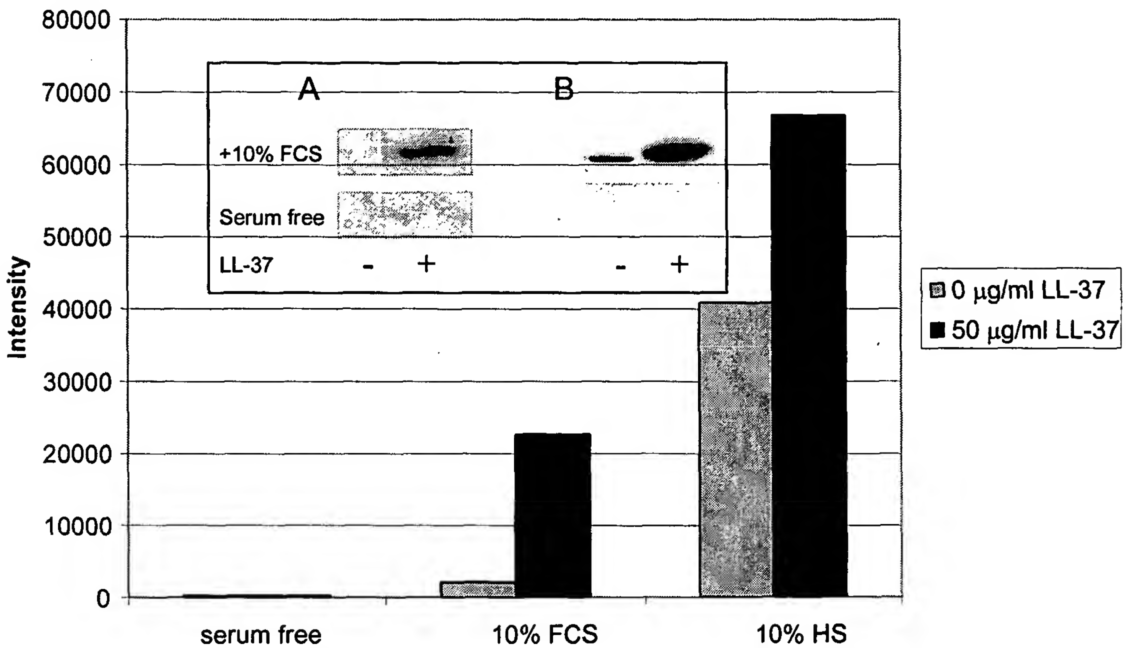


Figure 2. Exposure to SEQ ID NO: 1 induces phosphorylation of ERK1/2 and p38. Lysates from human peripheral blood derived monocytes were exposed to 50 μg/ml of SEQ ID NO: 1 for 15 minutes. A) Antibodies specific for the phosphorylated forms of ERK and p38 were used to detect activation of ERK1/2 and p38. All donors tested showed increased phosphorylation of ERK1/2 and p38 in response to SEQ ID NO: 1 treatment . One representative donor of eight. Relative amounts of phosphorylation of ERK (B) and p38(C) were determined by dividing the intensities of the phosphorylated bands by the intensity of the corresponding control band as described in the Materials and Methods.

Fig. 3. LL-37 induced phosphorylation of ERK1/2 does not occur in the absence of serum and the magnitude of phosphorylation is dependent upon the type of serum present. Human blood derived monocytes were treated with 50 μg/ml of LL-37 for 15 minutes. Lysates were run on a 12% acrylamide gel then transferred to nitrocellulose membrane and probed with antibodies specific for the phosphorylated (active) form of the kinase. To normalize for protein loading, the blots were reprobed with β-actin. Quantification was done with ImageJ software.
The ***Figure 3 inset*** demonstrates that LL-37 is unable to induce MAPK activation in human monocytes under serum free conditions. Cells were exposed to 50 mg/ml of LL-37 (+), or endotoxin free water (-) as a vehicle control, for 15 minutes. (A) After exposure to LL-37 in media containing 10% fetal calf serum, phosphorylated ERK1/2 was detectable, however, no



phosphorylation of ERK1/2 was detected in the absence of serum (n=3). (B) Elk-1, a transcription factor downstream of ERK1/2, was activated (phosphorylated) upon exposure to 50 μg/ml of LL-37 in media containing 10% fetal calf serum, but not in the absence of serum (n=2).

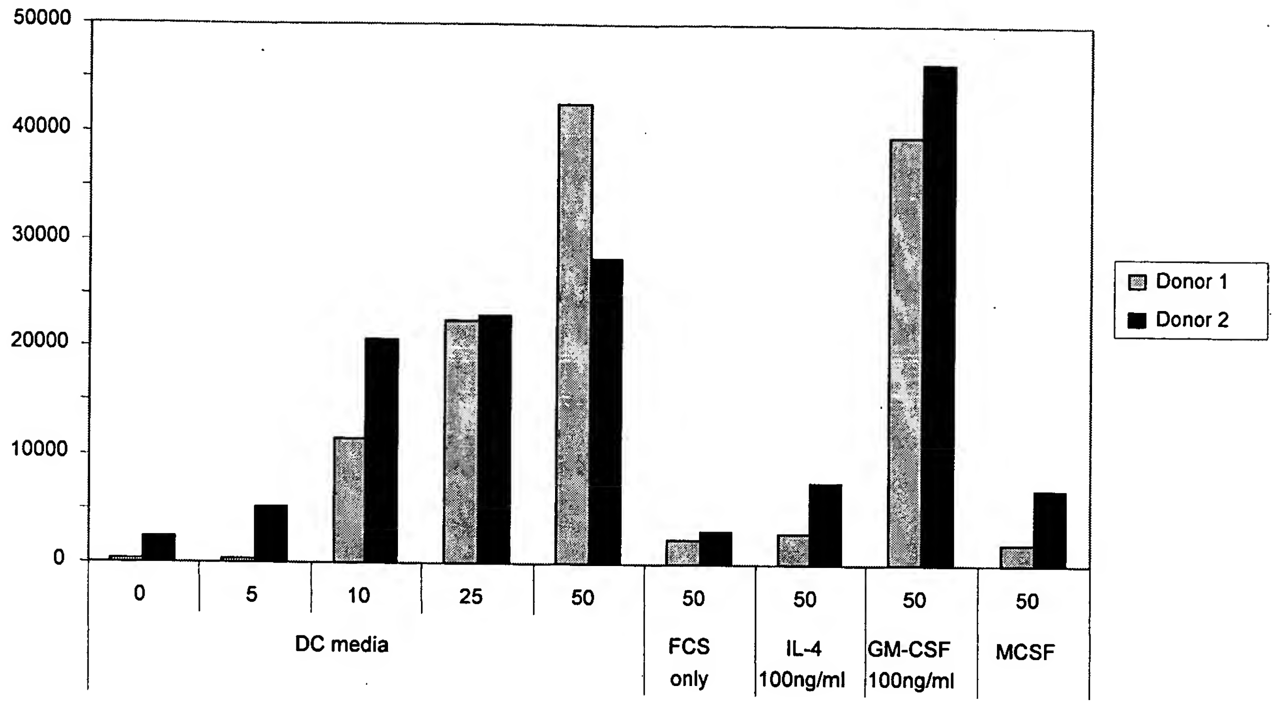
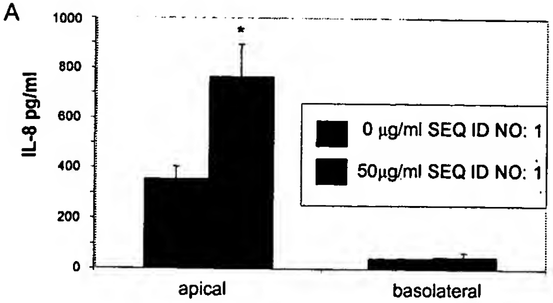


Figure 4. LL-37 induced activation of ERK1/2 occurs at lower concentrations and is amplified in the presence of certain cytokines. When freshly isolated monocytes were stimulated in media containing both GM-CSF (100ng/ml) and IL-4 (100ng/ml) LL-37 induced phosphorylation of ERK1/2 was apparent at concentrations as low as 5 μg/ml. This synergistic activation of ERK1/2 seems to be due primarily to GM-CSF.

A



B

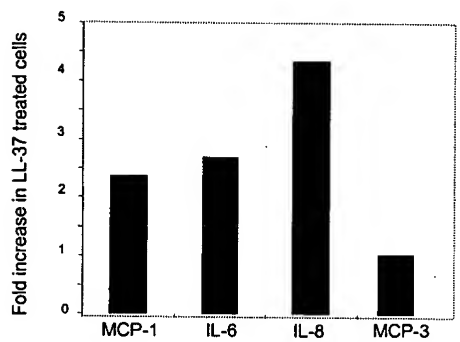


Figure 5. Peptide affects both transcription of various cytokine genes and release of IL-8 in the 16HBE4o- human bronchial epithelial cell line. Cells were grown to confluency on a semi-permeable membrane and stimulated on the apical surface with 50 μg/ml of SEQ ID NO: 1 for four hours. A) SEQ ID NO: 1 treated cells produced significantly more IL-8 than controls, as detected by ELISA in the supernatant collected from the apical surface, but not from the basolateral surface. Mean ± SE of three independent experiments shown, asterisk indicates p=0.002. B) RNA was collected from the above experiments and RT-PCR was performed. A number of cytokine genes known to be regulated by either ERK1/2 or p38 were up-regulated upon stimulation with peptide. The average of two independent experiments is shown.